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Designing, synthesis and spectral characterization of Schiff base transition metal complexes: DNA cleavage and antimicrobial activity studies

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Abstract: A new series of transition metal complexes of Cu(II), Ni(II), Co(II) and Zn(II) have been designed and synthesized using a Schiff base (L) derived from 4-aminoantipyrine, benzaldehyde and *o*-phenylenediamine. The structural features were derived from their elemental analyses, magnetic susceptibility and molar conductivity, as well as from mass, IR, UV–Vis, ^{1}H-NMR and ESR spectral studies. The FAB mass spectral data and elemental analyses showed that the complexes had a composition of the ML type. The UV–Vis and ESR spectral data of the complexes suggested a square-planar geometry around the central metal ion. The magnetic susceptibility values of the complexes indicated that they were monomeric in nature. Antimicrobial screening tests were also performed against four bacteria, *viz*. *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis* and three fungi, *viz*. *Aspergillus niger*, *Aspergillus flavus* and *Rhizoctonia bataicola.* These data gave good results in the presence of metal ion in the ligand system. The nuclease activity of the above metal complexes shows that only the copper complex cleaves CT DNA in the presence of an oxidant.

Keywords: 4-aminoantipyrine; benzaldehyde; *o*-phenylenediamine; CT DNA; Schiff base; antimicrobial activity.

INTRODUCTION

Schiff bases of 4-aminoantipyrine and their complexes have a variety of applications in biological, clinical, analytical and pharmacological areas.[1,2] Studies of new kinds of chemotherapeutic Schiff bases are now attracting the attention of biochemists.[3,4] Earlier works reported that some drugs showed increased activity when administered as metal complexes rather than as organic compounds. According to cell biology, deoxyribonucleic acid (DNA) is the primary target molecule for most anticancer and antiviral therapies. Investigations of the interaction of DNA with small molecules are basic work in the design of new types of pharmaceu-

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tical molecules. Since the chemical nuclease activity of transition metal complexes was discovered in the 1980s,[5–7] studying the interaction model and the mechanism of transition metal complexes with DNA and exploring the application of metal complexes in antineoplastic medication, molecular biology and bioengineering have been hotspots in recent years. Certain kinds of metal complexes when interacted with DNA could induce the breakage of DNA strands by appropriate methods. Thus, with cancer genes, after DNA strands are cleaved, the DNA double strands break. The replication ability of cancer gene is then destroyed. Ueda *et al*.[8] showed that the copper complex could cleave DNA in the presence of ascorbate or hydroquinone. It was suggested that the reductive capability of the reductants had a critical influence on DNA cleavage. The coordinating property of the 4-aminoantipyrine ligand was modified to give flexible ligand systems, formed by condensation with a variety of reagents, such as aldehydes, ketones, thiosemicarbazides, carbazides, *etc*.[9–19] A literature search revealed that no work has been undertaken on the condensation of 4-aminoantipyrine, benzaldehyde and *o*-phenylenediamine. Hence, in this paper, the synthesis, characterization, antimicrobial and DNA studies of transition metal complexes containing Schiff base derived from 4-aminoantipyrine, benzaldehyde and *o*-phenylenediamine are described.

EXPERIMENTAL

Syntheses of Schiff base (L)

An ethanolic solution (20 mL) of 1-phenyl-2,3-dimethyl-4-aminopyrazol-5-one (4-aminoantipyrine) (2.03 g, 0.010 mol) was added to an ethanolic solution of benzaldehyde (1.06 g, 0.010 mol). On stirring, a yellow-coloured solid (**I**) separated, which was filtered and recrystallised from ethanol. Compound **I** (2.9 g, 0.010 mol) was added to an ethanolic solution (20 mL) of *o*-phenylenediamine (0.54 g, 0.0050 mol). The mixture was refluxed for *ca.* 30 h after addition of anhydrous potassium carbonate. The potassium carbonate was filtered off from the reaction mixture and the solvent was evaporated. A dark yellow solid product (L) was separated, which was filtered and recrystallised from ethanol (Fig. 1).

Syntheses of complexes

A solution of metal(II) chloride in ethanol (2.0 mmol) was refluxed with an ethanolic solution of the Schiff base (2.0 mmol) for *ca.* 5 h. Then the solution was reduced to one-third of its volume on a water bath. The precipitated solid complex was filtered and washed thoroughly with ethanol and dried under vacuum.

Antimicrobial activity

The in vitro biological screening effects of the investigated compounds were tested against the bacteria: *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis* by the well-diffusion method, using agar nutrient as the medium. The antifungal activities of the compounds were evaluated by the well-diffusion method against the fungi, *viz*. *Aspergillus niger*, *Aspergillus flavus* and *Rhizoctonia bataicola* cultured on potato dextrose agar as medium. Stock solutions (10 mM) were prepared by dissolving the compounds in DMSO and the solutions were serially diluted in order to find the *MIC* values. In a typical procedure,[20] a well

was made on the agar medium inoculated with micro-organisms. The well was filled with the test solution using a micropipette and the plate was incubated, 24 h for bacteria and 72 h for fungi, at 35 °C. During this period, the test solution diffused and the growth of the inoculated micro-organisms was affected. The concentration at which an inhibition zone developed, was noted.

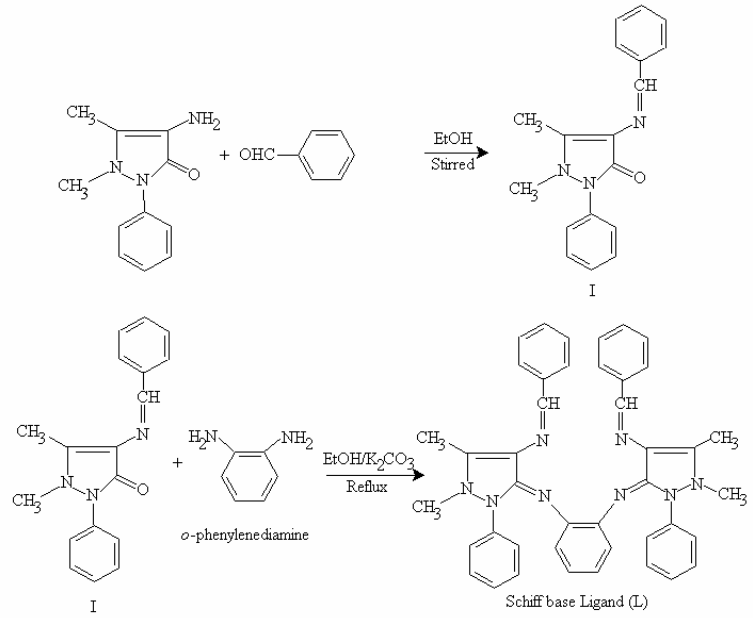

Fig. 1. Formation of Schiff base ligand (L).

Gel electrophoresis

The gel electrophoresis experiments were performed by incubation at 35 °C for 2 h as follows. The samples containing 30 μM calf thymus DNA (CT DNA), 50 μM of each complex and 50 μM H_2O_2 in 50 mM tris-HCl buffer (pH 7.2) were electrophoresed for 2 h at 50 V on a 1 % agarose gel using tris-acetic acid–EDTA buffer, pH 8.3. After electrophoresis, the gel was stained using 1.0 μg cm^{-3} ethidium bromide (EB) and photographed under UV light.

Apparatus and reagents

All reagents, 4-aminoantipyrine, benzaldehyde, *o*-phenylenediamine and various metal(II) chlorides were Merck products and used as supplied. Anhydrous grade ethanol, DMF and DMSO were purified according to standard procedures. Microanalytical data of the compounds were recorded at the Sophisticated Analytical Instrument Facility, Central Drug Research Institute (SAIF, CDRI), Lucknow. The FAB mass spectra of the ligand and its complexes were recorded at SAIF, Indian Institute of Technology, Mumbai. The IR spectra of the samples were recorded on a Perkin-Elmer 783 spectrophotometer in the 4000–400 cm^{-1} range using KBr pellets. The UV–Vis spectra were recorded on a Shimadzu UV-1601 spectrophotometer using DMF as the solvent. The X-band ESR spectra of the complex were recorded at 300 and 77 K at IIT, Mumbai, using tetracyanoethylene (TCNE) as the g-marker. Magnetic susceptibility measurements of the complexes were carried out on a Guoy balance using copper sulphate as the calibrant. The molar conductance of the complexes was measured using a Systronic conductivity bridge. Solutions of CT DNA in 50 mM NaCl/50 mM tris-HCl

(pH 7.2) gave the ratio of the UV absorbance at 260 and 280 nm, A_{260}/A_{280}, of *ca.* 1.8–1.9, indicating that the DNA was sufficiently free of protein contamination. The DNA concentration was determined by the UV absorbance at 260 nm after 1:100 dilution. The molar absorption coefficient was taken as 660 m^2 mol^{-1}. Stock solutions were kept at 4 °C and used after not more than 4 days. Doubly distilled H_2O was used to prepare the buffer. The antimicrobial activities of the ligand and its complexes were carried out by the well-diffusion method.

RESULTS AND DISCUSSION

The analytical data for the ligand and complexes together with some physical properties are summarized in Table I. The analytical data of the complexes correspond well with the general formula ML, where M = Cu(II), Ni(II), Co(II) and Zn(II); L = $C_{42}H_{38}N_8$. The magnetic susceptibilities of the complexes at room temperature were consistent with square planar geometry around the central metal ion. The higher conductivity values of the chelates support the electrolytic nature of the metal complexes.

TABLE I. Physical characterization, analytical, molar conductivity, Λ_m, and magnetic susceptibility data of the ligand and the complexes

Cmpd.	Molecular formula	Colour	M.p. °C	Found (Cacld.), %				Λ_m / Ω^{-1} cm^2 mol^{-1}	μ_{eff} / μ_B
				M	C	H	N		
L	$C_{42}H_{38}N_8$	Yellow	198	–	77.0 (77.0)	5.8 (5.9)	17.1 (17.2)	–	–
$[CuL]Cl_2$	$CuC_{42}H_{38}N_8Cl_2$	Black	260	8.8 (8.9)	70.2 (70.3)	5.3 (5.4)	15.6 (15.6)	94	1.73
$[NiL]Cl_2$	$NiC_{42}H_{38}N_8Cl_2$	Light green	272	8.2 (8.3)	70.7 (70.7)	5.4 (5.4)	15.7 (15.7)	98	–
$[CoL]Cl_2$	$CoC_{42}H_{38}N_8Cl_2$	Brown	242	8.2 (8.2)	70.7 (70.7)	5.4 (5.3)	15.7 (15.6)	93	3.52
$[ZnL]Cl_2$	$ZnC_{42}H_{38}N_8Cl_2$	Colour-less	265	9.2 (9.3)	70.0 (70.0)	5.3 (5.4)	15.6 (15.6)	82	–

The FAB mass spectra of the Schiff base and its complexes were compared for their stoichiometric compositions. The molecular ion peak for the ligand was observed at 654 *m*/*z*, which is also supported by the "nitrogen rule", since the compound possesses eight nitrogen atoms. For the copper complex, the molecular ion peak appeared at 791 *m*/*z*, which confirms the stoichiometry of metal complexes as being of the ML type. It is also supported by the mass spectra of other complexes. This composition is further supported by the microanalytical data (Table I).

The IR spectra provide valuable information regarding the nature of the functional group attached to the metal atom.[21] The spectrum of the free Schiff base ligand shows two –C=N bands in the region 1650–1565 cm^{-1}, which is shifted to lower frequencies in the spectra of all the complexes (1620–1530 cm^{-1}), indicating the involvement of the –C=N nitrogen in the coordination to the metal ion.[22] Coordination of the Schiff base to the metal through the nitrogen atom is expected to reduce the electron density in the azomethine link and lowers the

$\nu_{C=N}$. All the complexes show bands in 1090–1100 cm^{-1} and 700–750 cm^{-1} regions and can be assigned to phenyl ring vibrations.[23] Assignment of the proposed coordination sites is further supported by the appearance of medium bands at 450–500 cm^{-1}, which could be attributed to ν_{M-N}.[24,25]

The electronic absorption spectra of the Schiff base, Cu(II), Co(II), and Ni(II) complexes were recorded at 300 K. The absorption region, assigned and the proposed geometry of the complexes are given in Table II. Based on these data, a square-planar geometry was assigned to the complexes. These values are comparable with those of the other reported complexes.[26–29]

TABLE II. Electronic absorption spectral data of the compounds

Cmpd.	Solvent	Absorption, cm^{-1}	Band assignment	Geometry
L	DMSO	26525	ILCT	–
$[CuL]Cl_2$	DMSO	27397, 23752	ILCT, $^2B_1g \rightarrow {}^2A_1g$	Square-planar
$[CoL]Cl_2$	DMSO	27173, 19417	ILCT, $^1A_1g \rightarrow {}^1B_1g$	Square-planar
$[NiL]Cl_2$	DMSO	27027,25380, 19607	ILCT, $^1A_1g \rightarrow {}^1A_2g$, $^1A_1g \rightarrow {}^1B_1g$	Square-planar

The ESR spectra of the copper complex, recorded in DMSO solution at 300 and 77 K are shown in Figs. 2a and 2b. The frozen solution spectrum shows a well resolved four line spectrum and no features characteristic for a dinuclear complex. This is also supported by the magnetic moment of copper complex (1.73 μ_B) which confirms the mononuclear nature of the complex. The spin Hamiltonian parameters for the copper complex were calculated from the spectra. The g-tensor values of this copper(II) complex can be used to derive the ground state. In square-planar complexes, the unpaired electron lies in the $d_{x^2-y^2}$ orbital, giving $^2B_{1g}$ as the ground state with $g_{\|} > g_{\perp} > 2$, while the unpaired electron lies in the d_{z^2} orbital, giving $^2A_{1g}$ as the ground state with $g_{\perp} > g_{\|} > 2$. From the observed values, it is clear that $g_{\|}(2.17) > g_{\perp}(2.03) > 2$, which suggests that the complex is square planar. This is further supported by the fact that the unpaired electron lies predominantly in the $d_{x^2-y^2}$ orbital,[30–33], as was evident from the value of the exchange interaction term G, estimated from the expression:

$$G = \frac{g_{\|} - 2.0023}{g_{\perp} - 2.0023}$$

If $G > 4.0$, the local tetragonal axes are aligned parallel or only slightly misaligned. If $G < 4.0$, significant exchange coupling is present and the misalignment is appreciable. The observed value for the exchange interaction parameter for the copper complex ($G = 6.5$) suggests that the local tetragonal axes are aligned parallel or slightly misaligned and that the unpaired electron is present in the $d_{x^2-y^2}$ orbital. This result also indicates that the exchange coupling effects are not operative in the present complex.[34]

Based on the above spectral data, the structure of the complex given in Fig. 3 is proposed.

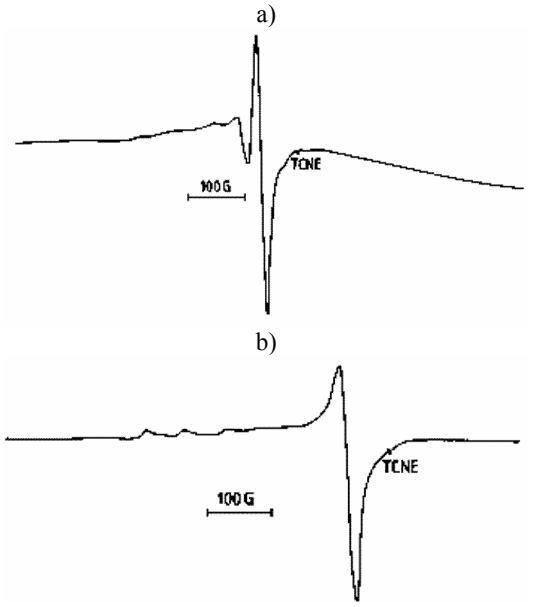

Fig. 2. ESR spectra of the copper complex at 300 (a) and 77 K (b).

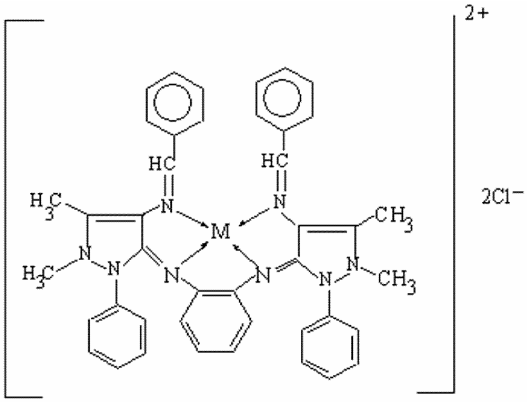

Fig. 3. The proposed structure of the Schiff base complexes.

For in vitro antimicrobial activity, the investigated compounds were tested against the bacteria *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis* and the fungi *Aspergillus niger*, *Aspergillus flavus* and *Rhizoctonia bataicola*. The minimum inhibitory concentration (*MIC*) values of the investigated compounds are summarized in Table III. From the table, the observed *MIC* values indicate that most of the complexes have higher antimicrobial activity than the free ligand. Such increased activity of the metal chelates can be explained on the basis of the chelation theory. On chelation, the polarity of the metal ion is reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the delocalization of the π-electrons over the whole chelate ring

and enhances the penetration of the complexes into lipid membranes and the blocking of the metal binding sites in the enzymes of micro-organisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which further restricts the growth of the organism.[18]

TABLE III. Antibacterial activity of the Schiff base ligand and its metal complexes

Cmpd.	*MIC* / 10^{-2} M						
	S. typhi	*S. aureus*	*E. coli*	*B. subtilis*	*A. niger*	*A. flavus*	*R. bataicola*
L	5.8	5.9	5.7	6.1	7.1	7.2	7.3
$[CuL]Cl_2$	4.6	4.4	4.6	4.8	5.8	5.9	6.1
$[CoL]Cl_2$	4.7	5.1	4.9	4.9	5.7	5.9	5.6
$[NiL]Cl_2$	4.8	4.9	4.6	4.7	5.4	5.7	5.8
$[ZnL]Cl_2$	4.9	4.8	4.7	4.8	6.0	5.9	6.1

The cleavage efficiency of the complexes compared to that of the control is due to their efficient DNA-binding ability. The metal complexes were able to convert supercoiled DNA into open circular DNA. The general oxidative mechanism is proposed to account for DNA cleavage by hydroxyl radicals *via* abstraction of hydrogen atoms from sugar units and predict the release of specific residues arising from transformed sugars, depending on the position from which the hydrogen atom is removed.[35] The cleavage was inhibited by free radical scavengers, implying that hydroxyl radical or peroxy derivatives mediate the cleavage reaction. The reaction was modulated by metallocomplexes bound hydroxyl radical or a peroxo species generated from the co-reactant H_2O_2.

In the present study, a CT-DNA gel electrophoresis experiment was conducted at 35 °C using the synthesized complexes in the presence of H_2O_2 as an oxidant. As can be seen from the results in Fig. 4, at very low concentration, only the copper complex exhibited nuclease activity in the presence of H_2O_2. The control experiment using DNA alone (lane 1) did not show any significant cleavage of CT-DNA, even with a longer exposure time. From the observed results, it can be concluded that the copper complex (lane 2), cleaves DNA as compared to the control DNA while the other complexes (lanes 3–5) do not cleave DNA in the presence of H_2O_2. Furthermore, the presence of a smear in the gel diagram indicates the presence of radical cleavage.[36]

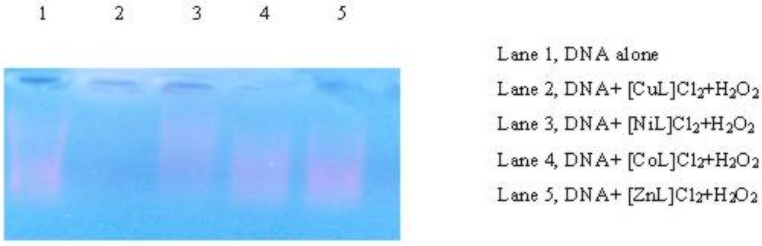

Fig. 4. Changes in the agarose gel electrophoretic pattern of CT-DNA induced by H_2O_2 and the metal complexes.

CONCLUSIONS

In this paper, the coordination chemistry of a Schiff base ligand, obtained from the reaction of 4-aminoantipyrine, benzaldehyde and *o*-phenylenediamine is described. Cu(II), Co(II), Ni(II) and Zn(II) complexes were synthesized using the above Schiff base ligand and characterized by spectral and analytical data. Based on these data, a square-planar geometry was assigned to the complexes. The metal complexes have higher antimicrobial activity than the ligand. The interaction of these complexes with CT-DNA was investigated by gel electrophoresis, from the results of which it was observed that the copper complex cleaved DNA in the presence of H_2O_2 as compared to the control DNA and the other complexes.

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И З В О Д

ДИЗАЈН, СИНТЕЗА И СПЕКТРАЛНА КАРАКТЕРИЗАЦИЈА ШИФОВИХ БАЗА ПРЕЛАЗНИХ МЕТАЛНИХ КОМПЛЕКСА: РАСКИДАЊЕ DNA И АНТИМИКРОБНО ПРОУЧАВАЊЕ

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Дизајнирана је серија комплекса прелазних метала Cu(II), Ni(II), Co(II) и Zn(II) и добијена помоћу Шифове базе (L) изведене из 4-аминопирина, бензалдехида и *o*-фенилендиамина. До структурних облика се дошло на основу њихових елементалних анализа, магнетне сусцептибилности, моларне проводљивости, масених, IR, UV–Vis, ^{1}H-NMR и ESR спектралних проучавања. FAB масени спектри и елементална анализа показали су да је састав комплекса ML типа. UV–Vis и ESR спектри комплекса сугеришу квадратно-планарну геометрију око централног металног јона. Вредности магнетне сусцептибилности комплекса указују на то да су они мономерне природе. Антимикробни тест је спроведен на четири бактерије, и то *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli*, и *Bacillus subtilis* и три гљивице, и то *Aspergillus niger*, *Aspergillus flavus* и *Rhizoctonia bataicola*. Тест је дао добре резултате у присуству металног јона у лигандном окружењу. Нуклеазна активност поменутих комплекса показује да само комплекс бакра раскида CT DNA у присуству оксиданта.

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REFERENCES

1. T. Hitoshi, N. Tamao, A. Hideyuki, F. Manabu, M. Takayuki, *Polyhedron* **16** (1997) 3787
2. T. Punniyamurthy, S. J. S. Kalra, J. Iqbal, *Tetrahedron Lett*. **36** (1995) 8497
3. Y. K. Choi, K. H. Chjo, S. M. Park, N. Doddapaneni, *J. Electrochem. Soc*. 142 (1995) 4107
4. B. Katia, L. Simon, R. Anne, C. Gerard, D. Francoise, M. Bernard, *Inorg. Chem*. **35** (1996) 387
5. D. S. Sigman, D. R. Graham, V. D. D'Aurora, A. M. Stern, *J. Biol. Chem*. **254** (1979) 12269
6. V. M. Downey B. R. Que, A. G. So, *Biochem. Biophys. Res. Commun*. **93** (1980) 264

7. E. L. Marshall, D. R. Graham, K. A. Reith, *Biochemistry* **20** (1981) 224
8. J. I. Ueda, M. Takai, Y. Shimazu, *Arch. Biochem. Biophys*. **357** (1998) 231
9. P. K. Radhakrishnan, *Polyhedron* **5** (1986) 995
10. R. C. Maurya, D. D. Mishra, M. Pandey, P. Shukla, R. Rathour, *Synth. React. Inorg. Met. Org. Chem*. **23** (1992) 167
11. R. K. Agarwal, P. Garg, H. Agarwal, D. Chandra, *Synth. React. Inorg. Met. Org. Chem*. **27** (1997) 251
12. L. Singh, N. Tyagi, N. P. Dhaka, S. K. Sindhu, *Asian J. Chem*. **11** (1999) 503
13. N. K. Jha, D. M. Joshi, *Synth. React. Inorg. Met. Org. Chem*. **16** (1986) 947
14. P. Thomas, G. Parmeshwaran, *J. Indian Chem. Soc*. **69** (1992) 117
15. A. P. Mishra, V. Srinivastava, S. K. Srinivastava, *Synth. React. Inorg. Met. Org. Chem*. **25** (1995) 21
16. R. Shankar, R. P. Kumar, S. K. Ramalingam, *Polyhedron* **5** (1986) 991
17. A. Kriza, A. Reiss, S. Florea, T. Caproiu, *J. Indian Chem. Soc*. **77** (2000) 207
18. N. Dharmaraj, P. Viswanathamurthi, K. Natarajan, *Transition Met. Chem*. **26** (2001) 105
19. N. Raman, A. Kulandaisamy, K. Jeyasubramanian, *Polish J. Chem*. **76** (2002) 1085
20. M. J. Pelczar, E. C. S. Chan, N. R. Krieg, *Microbiology*, 5th Ed., McGraw Hill Inc., New York, 1998
21. L. Heri, J. Taganine, B. M. Gupta, *Indian J. Chem*. **40A** (2000) 999
22. S. K. Sengupta, O. P. Pandey, J. K. Pandey, G. K. Pandey, *Indian J. Chem.* **40A** (2001) 887
23. K. Nakamoto, *Infrared and Raman Spectra of Inorganic and Coordination Compounds*, 3rd Ed., Wiley, New York, 1997
24. M. B. H. Howlader, M. S. Islam, *Indian J. Chem.* **46A** (2007) 440
25. M. Thankamony, K. Mohanan, *Indian J. Chem*. **46A** (2007) 247
26. M. F. Iskander, L. Ei-Syed, K. Z. Ismail, *Transition Met. Chem.* **4** (1979) 225
27. M. Thankamony, K. Mohanan, *Indian J. Chem*. **46A** (2007) 249
28. M. Thomas, M. K. M. Nair, R. K. Radhakrishan, *Synth. React. Inorg. Met. Org. Chem.* **25** (1995) 471
29. R. M. Kadam, M. D. Sastry, M. K. Bhide, S. A. Chavan, J. V. Yakhmi, O. Khan, *Chem. Phys. Lett*. **281** (1997) 292
30. M. Base, K. Ohta, Y. Babu, M. D. Sastry, *Chem. Phys. Lett*. **324** (2000) 330
31. R. K. Ray, G. B. Kauffman, *Inorg. Chim. Acta* **173** (1990) 207
32. K. Jeyasubramanian, S. A. Samath, S. Thambidurai, R. Murugesan, S. K. Ramalingam, *Transition Met. Chem*. **20** (1996) 76
33. A. M. F. Benial, V. Ramakrishnan, R. Murugesan, *Spectrochim. Acta* **56A** (2000) 2775
34. L. J. Boucher, E. C. Tyanan, T. F. Yen, *Electron Spin Resonance of Metal Chelates*, Plenum Press, New York, 1969
35. G. Prativel, M. Pitie, J. Bernadou, B. Meunier, *Angew. Chem. Int. Ed. Eng*. **30** (1991) 702
36. A. M. Thomas, A. D. Naik, M. Nethaji, A. R. Chakravarty, *Indian J. Chem*. **43A** (2004) 691.